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Concurrent outbreaks of influenza and parvovirus B19 in a boys' boarding school

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SUMMARY

In the spring term of 1985 there was a protracted outbreak of upper respiratory tract febrile illness consistent with a clinical diagnosis of influenza in a boys' boarding school, which lasted from 23 January to 29 March. Although influenza virus infection was confirmed in 89% of cases in the first half of the term, 53% of the cases which occurred in the second half of the term had no evidence of infection with influenza virus. Between 5 February and 31 March 28 boys presented with skin rashes consistent with a clinical diagnosis of erythema infectiosum; 68% of these were associated with parvovirus B19. Investigation of the cases of clinical influenza with no identified respiratory pathogen revealed a 58% infection rate with B19. B19 DNA was identified in either throat swabs or acute stage bloods of nine pupils with influenza-like symptoms.

Cohort studies revealed that 44% of pupils aged 15–16 years were immune before the outbreak compared with only 17% of pupils aged 11–12 years. Infection in the younger group was common and was associated with influenza-like illnesses as well as rashes. Forty-eight per cent of those who did not report any symptoms were also infected with B19.

INTRODUCTION

Previous studies in school-children have shown that during influenza outbreaks a clinical diagnosis of influenza is confirmed in the laboratory in approximately 90% of cases (1). In the spring term of 1985 there was an outbreak of clinical influenza-like illness at Christ's Hospital, a boarding school in Sussex which then numbered some 675 boys aged 11–18 years. The outbreak was unusually protracted for this community and lasted from 24 January until 29 March. (Influenza outbreaks usually last about 1 month in this school). Influenza viruses A and B were isolated from patients. The outbreak spanned a mid-term break (22–24 February) and during the second part of the term 53% of the investigated cases had no laboratory evidence of infection with influenza viruses. During February and March 28 pupils reported to the school medical officer (T.W.H.)

with rashes which were consistent with a clinical diagnosis of fifth disease or erythema infectiosum (EI). Nineteen of the 28 were shown to be infected with parvovirus B19. The unusual epidemiology of the influenza outbreak in conjunction with the outbreak of rashes stimulated us to investigate cases of influenza-like illness for evidence of infection with B19.

METHODS

Study population

Christ's Hospital is a boarding school in Sussex, which at the time of this study numbered 675 boys aged 11–18 years. The pupils were accommodated in 12 houses, 6 for senior boys aged 15 years and over, and 6 for junior boys aged 11–15 years. All pupils who are ill report to the school infirmary. Clinical diagnoses are the responsibility of the medical officer (T.W.H.) who maintains the medical records for each child.

Investigation of respiratory illness for respiratory pathogens

Throat swabs were collected from every pupil who reported to the school infirmary with respiratory illness. The swabs were transported to the laboratory in a transport medium which did not contain antibiotics. These were examined for viruses in cell culture (Madin–Darby canine kidney, baboon kidney and fibroblasts [MRC-5]) and for bacterial pathogens. In addition to throat swabs, naso-pharyngeal aspirates and acute blood samples were collected from pupils with a clinical diagnosis of influenza. These specimens were all collected within 3 days of onset of symptoms. The aspirates were examined for the presence of influenza viruses A and B, adenoviruses, respiratory syncytial virus (RSV) and para-influenza viruses 1 and 3 by indirect immunofluorescence (reagents obtained from Public Health Laboratory Service Division of Microbiological Reagents and Quality Control), and then cultured. Convalescent blood samples were collected in May 1985 from most pupils who had suffered an influenza-like illness but from whom influenza virus had not been isolated. Blood samples collected in May or October 1984 before the outbreak and again a year later were available from two groups of pupils who joined the school in the autumn of 1980 (seniors) and the autumn of 1984 (juniors). These groups formed part of a long term study of influenza at the school in which the children were bled annually, irrespective of clinical history, to provide an estimate of asymptomatic infection.

Paired serum samples from pupils with influenza-like illnesses were examined for antibodies to influenza viruses A H1N1, A H3N2 and B by radial haemolysis (2) using the following strains: A/Philippines/2/82 (H3N2); A/Chile/1/83 (H1N1); and B/USSR/2/82. The viruses were supplied by Dr. J. S. Oxford, National Institute for Biological Standards and Control, UK. Where there was no laboratory evidence of infection the sera were examined for evidence of infection with other respiratory viruses (adenovirus, RSV, parainfluenza viruses) and *Mycoplasma pneumoniae* by the complement fixation test using antigens supplied by the PHLS Division of Microbiological Reagents and Quality Control.

Investigation of respiratory illness for parvovirus B19

Throat swabs from which virus had not been isolated were examined for the presence of parvovirus B19 by dot-blot hybridization using a ^{32}P labelled cloned portion of virus genome (3).

Acute blood samples were examined for the presence of B19 virus by dot-blot hybridization. Acute and convalescent samples were examined for IgM and IgG class antibody using antibody capture radioimmune assay techniques employing a monoclonal antibody preparation labelled with ^{125}I (4).

Investigation of cases of erythema infectiosum

Blood samples were collected when the boys were first seen by the school medical officer. A further sample was collected during convalescence from those pupils for whom a diagnosis could not be made on the first sample. Acute blood samples were examined for evidence of parvovirus B19 infection by the detection of virus DNA and IgM class antibody. Acute and convalescent paired samples were examined for IgG class antibody. Convalescent samples were also examined for IgM class antibody where no acute sample was available.

The sera were also examined for evidence of infection with rubella virus by HI and μ-capture ELISA for IgM class antibody (Northumbria Biologicals). The immune status of all pupils to measles had been assessed on entry to the school. Any who were shown to be susceptible had been vaccinated before this outbreak.

RESULTS

Course of the outbreaks

Influenza. The course of the epidemic of influenza-like illness throughout the school is illustrated in Fig. 1*a*. There was a total of 206 cases. The first case occurred on 23 January and the last 2 months later on 29 March. The outbreak affected boys of all ages.

Erythematous rash illness. The course of the outbreak of erythematous illness is shown in Fig. 1*b*. The first child presented with a rash on 5 February. Between then and the end of term on 31 March there were a further 27 cases, 26 of which occurred during March. Ten of the cases occurred in senior boys and 18 in juniors.

Investigation of pupils with influenza-like symptoms

The distribution of the cases among senior and junior pupils in relation to onset of symptoms is shown in Table 1.

During the first half of the term there were 143 cases of influenza-like illness of which 137 were investigated in the laboratory. One hundred and twenty-two of these (89%) had evidence of infection with influenza viruses (Table 1, cols A and C) and influenza virus was isolated from 53% of these. The majority of cases (111) occurred in junior boys although seniors were also affected.

During the mid-term break there were a further 15 cases, 8 in junior boys and 7 in seniors. Five were investigated and all were confirmed as influenza virus infections by serology. (Isolation specimens were not available from pupils who were ill during the school break.)

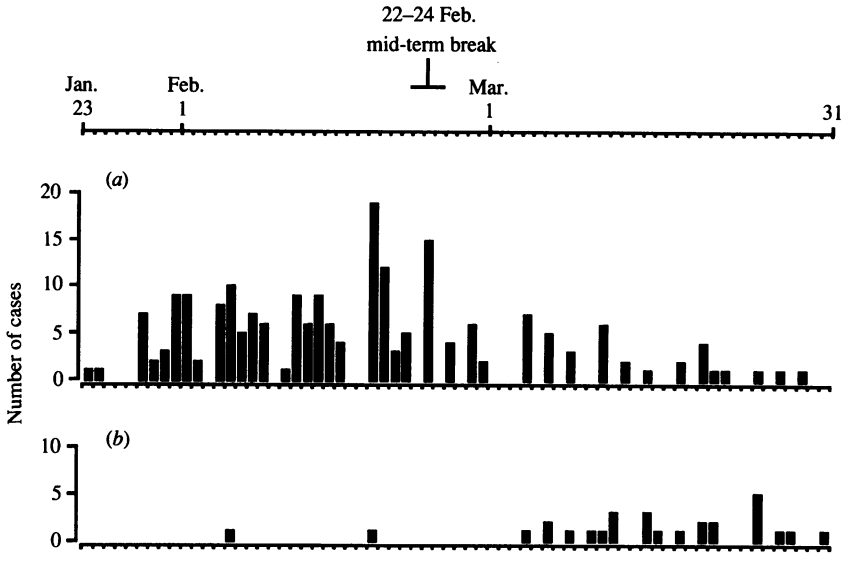

Fig. 1. The time-courses of the outbreaks of influenza-like illness (*a*) and erythematous rashes (*b*) in the school. The heights of the columns show the number of new cases diagnosed each day. Cases which occurred over the mid-term break are shown as one column on 23 February.

After the break there were a further 48 cases of influenza-like illness. Laboratory evidence of infection with influenza viruses remained high amongst senior pupils (15 out of 18 investigated – 83%). However of the 29 cases in juniors only 7 (24%) had evidence of influenza virus infection.

Twenty-four of the pupils who presented with influenza-like symptoms during the outbreak reported a second illness (Table 2). Fourteen of these pupils experienced two episodes of influenza-like illness and the remaining 10 suffered erythematous rashes in their second illness.

The 14 pupils with two episodes of respiratory illness comprised 4 seniors (S1–S4) and 10 juniors (J1–J10). Two of the four seniors had evidence of infection with both influenza A and B. The other two were infected with influenza B on the occasion of the second illness, but no agent was identified in respect of the first illness. The 10 junior boys all had evidence of infection with influenza B on the occasion of their first illnesses, 9 of which occurred during the early part of the term and one of which occurred during the mid-term break. None had evidence of infection with influenza viruses at the time of the second illness, all of which occurred after the break, but in 6 there was evidence of B19 infection. Group A streptococci were isolated from the throat swab of one boy during his second illness.

The 10 boys who had rashes after their respiratory illnesses comprised 2 seniors (S5 and S6) and 8 juniors (J11–J18). Both seniors and 6 of the 8 juniors had evidence of influenza B virus infection associated with their respiratory illness and one had evidence of B19 infection. Nine of the 10 rash illnesses occurred during the latter part of the term and all 9 were associated with B19 infection. The laboratory findings for all the rash illnesses in the school are described later (see 'Investigation of cases of rash illness').

Table 1. *Infection with influenza viruses and parvovirus B19 in pupils with influenza-like illnesses*

Onset of symptoms	Senior/Junior pupils	Total cases	Number investigated	(A) Number infected with influenza viruses only: VI+ (i)	(A) Number infected with influenza viruses only: sero only (ii)	(B) B19 only: DNA+ (i)	(B) B19 only: sero only (ii)	(C) Number with serological diagnosis of influenza and B19	(D) Number not infected with 'flu or B19
Pre	Senior	32	30	15	9	—	—	2	4
half-term	Junior	111	107	49	31	—	7	16	4*
	All	143	137	64	40	—	7	18	8
Half-term	Senior	7	3	—	3	—	—	—	—
break	junior	8	2	—	2	—	—	—	—
	All	15	5	—	5	—	—	—	—
Post	Senior	19	18	10	4	—	1	1	2*
half-term	Junior	29	29	2	3	9	6	2	7*
	All	48	47	12	7	9	7	3	9

* In each group indicated, one pupil infected with β-haemolytic streptococci, group A.

Table 2. *Clinical and laboratory diagnosis in pupils with two clinical illnesses*

Senior/ junior pupil	Clinical diagnoses	Pupil	1st illness Date	1st illness laboratory diagnosis*	2nd illness Date	2nd illness laboratory diagnosis
Senior	Influenza-like ×2	S1	24 Jan.	—	18 Mar.	B VI+
		S2	31 Jan.	A VI+	18 Feb.	B and B19 sero
		S3	31 Jan.	A VI+	18 Mar.	B sero
		S4	1 Feb.	—	18 Feb.	B VI+
	Influenza-like +rash	S5	31 Jan.	B sero	5 Feb.	—
		S6	25 Feb.	B sero	25 Mar.	B19 IgM
Junior	Influenza-like ×2	J1	28 Jan.	B VI+	15 Mar.	BHS group A
		J2	28 Jan.	B VI+	27 Mar.	—
		J3	31 Jan.	B VI+	4 Mar.	B19 sero
		J4	4 Feb.	B sero	21 Mar.	B19 DNA
		J5	11 Feb.	B sero	6 Mar.	B19 DNA
		J6	14 Feb.	B sero	8 Mar.	B19 DNA
		J7	14 Feb.	B VI+	13 Mar.	B19 sero
		J8	18 Feb.	B sero	6 Mar.	B19 sero
		J9	21 Feb.	B VI+	20 Mar.	—
		J10	half-term	B sero	4 Mar.	B19 DNA
	Influenza-like +rash	J11	28 Jan.	B VI+	6 Mar.	B19 IgM
		J12	5 Feb.	B sero	25 Mar.	B19 IgM
		J13	12 Feb.	B sero	18 Mar.	B19 DNA
		J14	12 Feb.	B sero	25 Mar.	B19 DNA
		J15	13 Feb.	—	25 Mar.	B19 IgM
		J16	25 Feb.	B VI+	25 Mar.	B19 DNA
		J17	4 Mar.	B sero	11 Mar.	B19 IgM
		J18	20 Mar.	B19 DNA	27 Mar.	B19 IgM

* A, influenza A; B, influenza B; B19, parvovirus B19; BHS group A, β haemolytic streptococci group A; —, no agent identified; VI+, virus isolated; DNA, B19-specific DNA detected; sero, serological (antibody) evidence alone of infection — IgM specified where relevant to the diagnosis.

All cases of influenza-like illness which had been investigated for influenza virus infection but where no evidence of infection had been found, were investigated for evidence of B19 infection (Table 1, cols B and D). These comprised 15 cases during the first half of the term, 11 of which ocurred in junior boys, and 25 cases during the second half of the term, 22 of which occurred in junior boys. Evidence of B19 infection was found in 7 of the early cases and in 16 of the later ones. In the early cases the diagnosis was made by detecting antibody seroconversions, but in 9 of the later cases B19 virus DNA was detected either in the acute phase blood (7) or in the throat swab (2). All of these 9 were in junior boys, 4 of whom had had 2 episodes of influenza-like illness (J4–6 and J10 – see Table 2) and one of whom went on to develop a rash 7 days later at which time B19 specific IgM was detected in his blood (J18).

Cases of influenza virus infection which had been diagnosed only by antibody tests were also investigated for evidence of B19 infection (Table 1, cols A(ii) and C). During the early part of the term 58 cases had been diagnosed only by serology: 18 (31 %) of these also had evidence of B19 infection (col C). None of the

Table 3. *Laboratory diagnosis of rash illnesses*

Onset of symptoms	Senior/ junior pupils	Total cases	Number infected with rubella virus only (IgM)	Number infected with parvovirus B19 only		Number not infected (no agent identified)
				DNA	IgM	
Pre half-term	Senior	1	—	—	—	1
	Junior	1	1	—	—	—
	All	2	1	—	—	1
Post half-term	Senior	9	2	1	4	2
	Junior	17	—	7	7	3
	All	26	2	8	11	5

5 cases which were confirmed serologically as influenza virus infection during the mid-term break had evidence of recent B19 infection. During the latter part of the term there were 10 boys with serological evidence alone of influenza virus infection: 3 of these also had evidence of recent B19 infection. B19 virus DNA was not detected either in throat swabs or acute phase bloods in any of the 21 cases with serological evidence of both influenza virus and B19 virus infections.

Investigation of cases of rash illness

The laboratory diagnoses of all cases of rash illness are shown in Table 3. Three of the 28 cases were associated with rubella infection – two seniors and one junior. Of the remaining 25, 19 (76%) had evidence of infection with parvovirus B19. These comprised 5 senior boys and 14 juniors. All the B19 infections occurred after the mid-term break. Eight of the cases had B19 DNA present in the acute phase blood and in the remaining 11, B19-specific IgM was detected. There were no cases where concurrent infection with rubella and B19 was diagnosed. No causative agent was found for the remaining 6 cases (3 seniors and 3 juniors).

Cohort studies in senior and junior boys

Two cohorts, one in the senior part of the school and one in the junior part of the school, participated in the regular serological surveillance of influenza virus infection irrespective of the presence of clinical symptoms of influenza. From these children blood samples were collected annually and thus sera were available which spanned the outbreaks during the spring term of 1985. These sera were examined for antibodies to influenza virus and B19 virus and the results correlated with any reported symptoms during the spring term.

Pupils suffering from a variety of minor afebrile ailments (including headaches, coryza, non-streptococcal sore throats) attend the infirmary. Throat swabs are taken from these patients but they are not usually investigated further. They do not form a cohesive group as far as clinical diagnosis is concerned and in the analyses that follow these pupils have been included with those with no reported symptoms in the category 'no significant symptoms'. They comprise 13 of this category in the senior cohort (Table 4) and 17 in the junior cohort (Table 5).

Eighty-eight senior cohort boys were investigated (Table 4). Overall 49 (56%) were infected with influenza virus and 17 (19%) with B19. A high proportion (39

Table 4. *Infection with parvovirus B19 and influenza virus in senior cohort*

Clinical symptoms	Total in group	Infected with: Influenza virus only	B19 only	Influenza and B19	Neither
Influenza-like					
(i) One episode	12	8	—	1	3 (1 BHS*)
(ii) Two episodes	3	2 (pupil S1 and S3)†	—	1 (pupil S2)	—
Influenza-like and rash	1	—	—	1 (pupil S6)	—
Rash only	7	2 (1 rubella)	3	1	1 (rubella)
No significant symptoms	65	26	3	7	29
Total	88	38	6	11	33

* BHS, β-haemolytic streptococci, group A.
† See Table 2.

boys, 44%) had antibody and were therefore immune to B19 before the outbreaks; the seroconversion rate among the susceptibles was 35% (17 out of 49).

Sixteen boys had influenza-like illnesses. Only 3 of these 16 boys had evidence of infection with B19 including pupil S6 who also had a rash. In contrast 13 of these 16 boys (81%) had evidence of infection with influenza viruses. Virus was isolated from 7 of the 13, the remaining diagnoses being confirmed by serology.

Altogether 8 boys presented with rashes. Two were associated with rubella virus infection and 5 of the remainder had evidence of recent B19 infection. In the whole senior cohort the infection rate with B19 was highest among boys with rashes.

Sixty-five boys (74% of the cohort) did not report any significant symptoms. Fifty-one per cent (33 boys) of this group were infected with influenza virus and 15% (10 boys) with B19.

Ninety junior cohort boys were investigated (Table 5). Overall 53 (59%) were infected with influenza virus and 54 (60%) with B19. Only 15 (17%) of the group were immune to B19 before the outbreaks and the seroconversion rate among the susceptibles was 72% (54 out of 75).

There was a high incidence of influenza-like illness in this cohort; 42 boys (47% of the cohort) presented with such symptoms, 4 of whom also reported rashes on separate occasions. Among the 38 boys whose only symptoms were influenza-like, 26 (68%) had evidence of infection with B19, a significantly higher rate than in those with no history of illness ($0{\cdot}025 < P < 0{\cdot}05$). B19 DNA was detected in the throat swab of one boy (J6) collected during his second influenza-like illness. Thirty-three (79%) of the 42 boys with influenza-like symptoms had evidence of influenza virus infection. Virus was isolated from 15 of the 33, the remaining diagnoses being confirmed serologically.

All 6 boys who presented with rashes had evidence of B19 infection with B19 specific IgM detected.

Over half the cohort had no reported symptoms or only minor symptoms.

Table 5. *Infection with parvovirus B19 and influenza virus in junior cohort*

Clinical symptoms	Total in group	Infected with: Influenza virus only	B19 only	Influenza and B19	Neither
Influenza-like					
(i) One episode	34	11	7	15	1
(ii) Two episodes	4	—	—	4 (pupils J3,6–8)*	—
Influenza-like and rash	4	—	1 (pupil J15)	3 (pupils J11, 12,17)	—
Rash only	2	—	1	1	—
No significant symptoms	46	12	15	7	12
Total	90	23	24	30	13

* See Table 2.

Forty-one percent of this group had evidence of infection with influenza virus and 48% with B19. These high infection rates indicate how common was infection with both viruses in this age group.

DISCUSSION

The concurrent outbreaks of influenza and B19 infection in this population provided an opportunity to investigate the characteristics of infection with these agents in this community. Influenza virus infection in this population has been studied extensively for many years and various aspects of the epidemiology and outcome of infection with different serotypes have been published (5–7).

In this study the early part of the outbreak of influenza illness was clearly associated with infection with influenza virus and the clinical diagnosis was confirmed in 89% of cases: a rate comparable to other outbreak investigations at this school and others. After the mid-term break the proportion of influenza-like illnesses with a laboratory diagnosis of influenza virus infection fell to 47% and for 12 of the pupils who reported sick this was their second illness. At the same time as this outbreak of influenza-like illness with no evidence of influenza virus infection there was an outbreak of rash illnesses clearly associated with parvovirus B19. The association between B19 and the clinical presentation of EI has been well proven (8,9). An unusual feature of this study was the detection of B19 DNA in blood samples from eight of the patients collected up to 4 days after the appearance of the rash; virus has only rarely been detected after the onset of rash symptoms in adults.

Whilst the cohort studies showed a high incidence of B19 infection in pupils with rashes these studies also revealed that, particularly in the younger boys, where the incidence of infection with B19 was high overall (60%), many infections were not associated with rashes. Investigation of 40 cases throughout the school of influenza-like illness where there was no laboratory evidence of influenza virus infection revealed that 23 had evidence of B19 infection. Fifteen of these 23 cases occurred after the mid-term break in the same period as the outbreak of rash

illnesses. In view of the high incidence of infection, particularly in the younger boys where the majority of the unconfirmed respiratory illness occurred, it was important to establish a causal relationship beween infection and symptoms. In support of this B19 DNA was detected in nine of the cases at the time of the respiratory symptoms: for two B19 DNA was present in the throat swabs and the remaining seven had DNA in the acute stage blood sample. Additional evidence of the association was provided by the cohort studies where the incidence of B19 infection in the younger boys with influenza-like symptoms was higher than in those with no symptoms.

There were, in both senior and junior cohorts, some pupils with evidence of infection with both viruses. Among the children who did not report any significant symptoms the rate of dual infection was no more than would be expected on the basis of infection rates with the individual agents.

A proportion of the cases of influenza-like illness with serological evidence of influenza virus infection but from whom no virus was isolated also had serological evidence of B19 infection. The 21 cases which fell into this category were not randomly distributed: the majority occurred before the mid-term break when influenza virus infection was common. There was no evidence from DNA studies that B19 virus was present in the school at this time and the most likely explanation is that the B19 infections actually occurred during the second part of the term, and were largely asymptomatic.

A prodromal illness resembling influenza-like symptoms has been described preceding the onset of EI (9, 10). In this study only one child who had an influenza-like illness, at which time B19 DNA was detected in this acute blood sample, went on to develop a rash one week later coincident with the appearance of B19-specific IgM in his serum. He was not infected with influenza virus.

The increasing awareness of parvovirus B19 as a human pathogen has led to increasing numbers of positive diagnoses in recent years (10). The range of clinical presentations associated with infection has expanded to include mild respiratory symptoms as well as rashes and acute onset transient anaemia in those with predisposing factors. This study among 11- to 18-year-old boys has shown that whilst infection may result in clinical EI, approximately half of those infected may have no significant symptoms and others may suffer an influenza-like illness.

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